A typical parameter used for describing the resolving capability of a fluorescence microscope is the full width at half maximum (FWHM) obtained by fitting a Gaussian curve to the point spread function (PSF) generated by a fluorescent bead.
In this work we used a custom-built video-rate confocal microscope at the diffraction limit to measure variations of PSF FWHM as a function of PSF intensities at different photomultiplier (PMT) sensitivities. Images were obtained by summation (128-256) of pixels with 62.5 ns dwell time. We used fluorescence beads and fluorescence-conjugated IgGs excited at 488 and 561 nm with emission recorded centered at 536 nm and 607 nm, respectively. In general, FWHM as a function of PSF peak intensity remains invariant using maximum photomultiplier (PMT) sensitivity. On the other hand, when using lower PMT sensitivities, FWHM values were correlated with PSF intensities, being at the diffraction limit.
In agreement with this finding, we found that at a lower PMT sensitivity the image quality of cells (e.g. isolated heart myocytes labeled with anti-L-type Ca2+ channel and anti-ryanodine receptors) is greatly improved. These proteins accumulate along the tubular transversal structure. It was remarkable that when using high pixel sensitivity (25-16 nm/pixel) we could discriminate discrete molecular clusters near the diffraction limit (~250 nm). In summary, proper usage of PMT sensitivity helps revealing more subcellular structures using a multi-color laser-scanning fluorescence microscope.

3294-Pos Board B341
Infrared-Nanoscopy of Surface Patterns in Mixed Polymer Brushes
Marlena Filimon.
Ruhr University Bochum, Bochum, Germany.
Polymer system in their diversity may offer a range of alternative, especially in the form of suitably designed thin films. Thin films made of so-called polymer brush show unique properties that allow changing their topography between different morphologies. Mixed polymer brushed consisting of two homopolymers (polymethyl methacrylate and polystyrene) being covalently with one end to a solid substrate have attached abiding interest because of their ability to switch properties such as the surface energy and/or surface topography in response to challenge of their environment.[1] For this reason, a conventional microscopic technique (AFM or STM) cannot provide information on specificity of biomolecular interaction.
A near field microscope incorporating vibrational spectroscopy as a contrast mechanism would allow chemical mapping in the so called ''fingerprint region'', with the high spatial resolution of SNOM [2-4]. Using a scattering scanning near-field microscope (s-SNIM) allows us to simultaneously record topography and frequency-dependent near-field signal of organic and biological samples with sub-diffraction limited resolution of up to 90 nm [4]. For chemical imaging of surface patterns in mixed polymer brushes, we used two tunable lasers, a CO laser (4.8μm - 6.3μm) and a high power continuous wave infrared optical parametric oscillator (OPO) (3.2μm - 4.1μm) as radiation source. We performed measurements around 1740 cm-1 (C=O stretching mode of methyl methacrylate) and 2930cm-1 (C-H stretching mode of styrene). An advanced image processing of the topography and the near-field image provided the evaluation of frequency dependent contrast showing spectroscopic signature.
[1] S. Santer, Macromolecules, 39, 3056-3061, 2006
[2] L. Novotny, S. Stranick, Annu.Rev.Phys,Chem, 57, 303-331, 2006.
[3] B. Dragnea, Encyclopedia of Nanoscience and Nanotechnology, Dekker, 2703-2712, 2004.
[4] B. Knoll, F. Keilmann, Nature, 399 (6732), 134-137, 1999.
[5] I. Kopf et. al. J.Phys.Chem. C, 111, 8166-8171, 2007.

3295-Pos Board B342
A Scheme for Increasing the Collection Efficiency of Multiphoton Microscopy
Jesse D. McMullen, Warren Zipfel.
Cornell University, Ithaca, NY, USA.
Conventional widefield microscopy is hindered in thick specimens by the scattering of the light from the focal plane. By confining the source of fluorescence to a known volume, multiphoton microscopy turns all emitted light into potential signal. Most microscope objective lenses currently in use for two-photon microscopy are designed along conventional widefield imaging paradigms where rejection of scattered rays is critical. For this reason, collection of highly scattered light by typical objective lenses can be inefficient. To help increase collection in highly scattering samples we demonstrate a collar of light pipes that can be added to existing physiological objectives to dramatically increase the net fluorescence collected. Unlike other schemes, such as the use of parabolic reflectors, this setup is usable for *in vivo* imaging of animals and thick tissue explants.
Figure - Images of a hypocampal YFP slice imaged via epi-fluorescence through a 20x Olympus objective and through our collection collar.

3296-Pos Board B343
Monitoring Voltage-dependent Protein Dynamics Using Fringe-field Electric Impedance Tomography
Sameera Dharia, Gregory Dittami, Jeffery Wyrick, Richard Rabbitt.
University of Utah, Salt Lake City, UT, USA.
Fringe-field Electric Impedance Tomography (ff-EIT) is a non-invasive electrophysiological technique that monitors the local dielectric properties around resting and excited cell membranes. In our implementation of ff-EIT, a *Xenopus Oocyte* is positioned between a circumferentially distributed array of gold electrodes and is simultaneously excited using whole-cell voltage clamp. Radio frequencies (10 kHz-10 MHz) are passed between pairs of electrodes around the *Oocyte*. Data collected using the ff-EIT system can be used to temporally resolve the electrical response of the membrane during cellular excitation. *Xenopus Oocytes* were selected as a model cell to be used in the ff-EIT system due to their large size, visually polarized hemispheres, and ability to express exogenous membrane-bound proteins on their own membranes. Measurements made during cellular excitation show a significant difference in impedance change between either hemisphere of a native *Oocyte,* thereby demonstrating the ability of the ff-EIT system to record with subcellular resolution. Furthermore, results collected from *Oocytes* injected with potassium (Shaker) ion-channels indicate that the ff-EIT system can be used to sense 1) the voltage dependence of membrane-impedance change associated with ion-channel activation and 2) kinetic information associated with ion-channel inactivation. Preliminary data also suggests the potential use of ff-EIT in detecting voltage-sensor movement and in monitoring dielectric changes due to the various conformational states of voltage-sensitive proteins.

3297-Pos Board B344
Theoretical Limits To Errors, Acquisition Rates, And Resolution In Microscopy Of Switchable Fluorophores: Replacing The Diffraction Limit With The Algorithm Limit
Alexander R. Small, Forrest Hippensteel.
California State Polytechnic University, Pomona, Pomona, CA, USA.
We calculate error rates, and their effects on imaging speed and resolution, in techniques that overcome the diffraction limit by using switchable fluorescent molecules. Recent experimental work has beaten the diffraction limit in fluorescence microcopy by activating and localizing subsets of the fluorescent molecules in the specimen, and repeating this process until all of the molecules have been imaged. Examples include PhotoActivation Localization Microscopy (PALM), STOchastic Reconstruction Microscopy (STORM), and microscopy of blinking quantum dots. In all these techniques there is a tradeoff between speed (activating more molecules per imaging cycle) and error rates (activating nearby molecules and producing overlapping images that hide information on molecular positions), and so intelligent image-processing approaches are needed to identify and reject bright spots containing multiple molecules. We show that there is a maximum acquisition rate determined by this trade-off, and that how closely one can approach this acquisition rate depends on the capabilities of the algorithm used to distinguish single-molecule spots from multi-molecule spots. In particular, we calculate the error rates of commonly-used algorithms that use the shape of the bright spot rather than the overall intensity. This technique is used in STORM because fluorescent dyes have fluorescence efficiencies that can be strongly affected by the local environment. We show that the capabilities of these algorithms, in combination with the target contrast between fluorophores and background, determine whether the resolution is limited by the capabilities of the algorithm or the number of photons collected, leading to photon-limited and algorithm-limited resolution regimes. Finally, we consider algorithms that can infer molecular positions from images of overlapping blurs, and derive the dependence of the minimum acquisition time on algorithm performance for this class of algorithms.

3298-Pos Board B345
High-speed Super-resolution Imaging through Interpolated Deconvolution of Live-cell TIRF Images
Shaohui Huang, Lawrence Lifshitz, Karl Bellve, Clive Standley,
Kevin Fogarty, Michael Czech.
University of Massachusetts Medical School, Worcester, MA, USA.
Recent progresses in overcoming the diffraction-limited optical resolution have mostly relied on spatial modulation of fluorophore's distribution between its bright and dark photophysical states. Such feat is often accomplished through complex and expensive experimental setups, and almost all the related super-resolution imaging techniques are hostile towards live-cell studies. In contrast, super-resolution imaging through interpolated deconvolution (Carrington et al., 1995, Science, 268:1483) is a post-acquisition image-processing technique that is independent on microscope platform. Its efficient collection and utilization of fluorescence photons also make the technology potentially a preferred method

for live-cell imaging. However, performance of the technique is context-dependent: e.g., weak fluorescence signals and clustered sub-resolution structures typically yield poor deconvolution results. We have evaluated such difficulty using realistically simulated TIRF images of GLUT4 glucose transporters in cultured adipocytes, whose average diameter of 75nm is far below the optical resolution. An essential image-processing step isolating regions of high information content from a TIRF image was discovered, which enables subsequent deconvolution resolution approaching 100nm. Detailed analysis of deconvolution results as a function of signal-to-noise qualities of the original images suggests that super-resolution details can be resolved with TIRF images of live cells acquired at speed up to 10 fps.

3299-Pos Board B346

Trimming the resolution gap in the study of molecular and cellular events by means of High Data Output and automated three-dimensional Correlative Light-Electron Microscopy approach

Alberto Diaspro[1,2], Paolo Bianchini[1,2], Giuseppe Vicidomini[3,1], Katia Cortese[4,2], Maria C. Gagliani[4,2], Clara Santangelo[4,2], Patrizia Boccacci[3], Carlo Tacchetti[4,2].
[1]LAMBS MicroScoBio, Department of Physics - University of Genoa, Genoa, Italy, [2]IFOM - Foundation FIRC Institute for Molecular Oncology, Milan, Italy, [3]MicroScoBio, DISI - University of Genoa, Genoa, Italy, [4]MicroScoBio, DIMES - University of Genoa, Genoa, Italy.

Correlative light/electron microscopy (CLEM) allows the simultaneous observation of a given subcellular structure by fluorescence light microscopy (FLM) and electron microscopy. The use of this approach is becoming increasingly frequent in molecular and cellular biophysics. Here we report on a new high data output three-dimensional (3D) CLEM method based on the use of cryosections (Vicidomini et al.,Traffic, 2008). We successfully applied the method to analyze the structure of rough and smooth Russell bodies used as model systems. The major advantages of this approach are the following: (i) the ability to correlate several hundreds of events at the same time, (ii) the possibility to perform 3D correlation, (iii) the potential to immunolabel both endogenous and recombinantly expressed proteins at the same time and (iv) the effective combination of the high data analysis capability of FLM with the high precision-accuracy of transmission electron microscopy in a CLEM hybrid morphometry analysis. We have identified and optimized critical steps in sample preparation, defined routines for sample analysis and retracing of regions of interest, developed software for semi/fully automatic 3D reconstruction and defined preliminary conditions for an hybrid light/electron microscopy morphometry approach. The relevance of the presented approach is further enhanced by two important key elements, namely: the development of optical nanoscopy methods and the potentiality for exploring different correlative frameworks like optical nanoscopy vs. optical microscopy adding scanning force microscopy techniques.

3300-Pos Board B347

Seeing Multifunctional Nano- and Micro-particles Suitable for Imaging & Therapy Using Freeze-fracture Electron Microscopy

Brigitte Papahadjopoulos-Sternberg.
NanoAnalytical Laboratory, San Francisco, CA, USA.

The potency of nano- and micro-particles, loaded with therapeutic and/or diagnostics, is frequently depending upon their morphology adopted in a biological relevant environment. Freeze-fracture electron microscopy (ff-em) as a cryofixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, and imaging molecules[1-3]. At a resolution limit of 2 nm we are able to study the fate of such carriers related to their pay load, application milieu [4], and during their interaction with cells.
Using ff-em we studied the morphology of a wide variety of nano- and micro particles suitable as carriers for diagnostics as well as therapeutics including quantum dots (free and coupled to drug-loaded immunoliposomes), micelles (spherical-, disc-, and worm-type micelles) [5], small unilamellar liposome [6], multilamellar liposome, niosomes [7], cationic liposome/DNA complexes [8,9], polymer- or lipid-stabilized gas bubbles [10], cochleate cylinder, depofoam particles, and drug crystals. Recently we explored liposome-, virosome-, and virus-based vaccines, including measles vaccine powders, by ff-em.
[1] B. Sternberg, Liposome Technology, CRC Press I (1992) 363.
[2] B. Sternberg, Handbook Nonmedical Applications of Liposomes CRC Press (1996) 271.
[3] B. Sternberg, Medical Applications of Liposomes, Elsevier (1998) 395.
[4] I. Jääskeläinen et al. Int. J. Pharm. 167 (1998) 191.
[5] V. P. Torchilin et al. PNAS (2003) 100 (4) 1972.
[6] V. P. Torchilin et al. PNAS (2003) 100 (10) 603.
[7] B. Sternberg et al. Nature 378 (1995) 21.
[8] B. Sternberg et al. FEBS-Letters 356 (1994) 361.
[9] B. Sternberg et al. Biochim. Biophys. Acta 1375 (1998) 1375.
[10] C. Brancewicz et al. J. Disp. Sci. & Techn. 27:5 (2006) 761.

3301-Pos Board B348

New Approach To Quantitative Subcellular Imaging Of Phosphorus And Calcium Using Energy-filtered Transmission Electron Microscopy And Tomography

Maria A. Aronova[1], Youngchan Kim[2], Alioscka A. Sousa[1], Guofeng Zhang[1], Richard D. Leapman[1].
[1]NIBIB, NIH, Bethesda, MD, USA, [2]NIDDK, NIH, Bethesda, MD, USA.

Analytical electron microscopy provides high-resolution distributions of chemical elements by measuring characteristic core-edge signals that originate from interaction of the incident electrons with a thin section of a cell. Such elemental images give information about the organization of specific biomolecules within cellular organelles, as well as the distribution of ions involved in regulation of cellular processes. For example, mapping phosphorus enables visualization of nucleic acid, and calcium provides distributions of a major intracellular second messenger. Elemental mapping at ~10 nm spatial resolution is typically achieved using energy-dispersive x-ray spectroscopy (EDXS) or electron energy loss spectroscopy (EELS) in a scanning transmission electron microscope (STEM). We have developed a complementary approach based on energy-filtered transmission electron microscopy (EFTEM). It is demonstrated that quantitative 2D elemental distributions containing ~10^6 pixels can be obtained from large regions of cells, and that 3D elemental distributions can be obtained when EFTEM is combined with electron tomography. It is found that an accurate elemental distribution can be derived from just two energy-selected images, above and below a core-edge. However, since the core-edge signals for elements like calcium and phosphorus are relatively weak, it is important to model the spectral background carefully by correcting for plural scattering. We have applied quantitative ETFEM imaging and tomography to determine the 3D distributions of DNA in the cell nucleus, and to measure calcium in mitochondria of neurons.

3302-Pos Board B349

Investigating the Protective Effects of Milk Phospholipids Against Ultraviolet Exposure Using Confocal Reflectance Microscopy

Lily Laiho, Guillaume Brisson, Sophie Gallier, Rafael Jimenez-Flores.
California Polytechnic State University, San Luis Obispo, CA, USA.

Current research on bioactive molecules in milk have documented health advantages of bovine milk and its components. Milk phospholipids, selected for this study, represent molecules with great potential benefit in human health and nutrition. In this study we used confocal reflectance microscopy to monitor changes in skin morphology upon exposure to ultraviolet light and evaluate the potential of milk phospholipids in preventing photodamage to skin. We imaged skin equivalent models based on human keratinocytes and dermal fibroblasts cultured in a collagen matrix. We compared images from skin equivalent models with (a) no exposure to UV light, (b) exposure to a dose of 60 mJ/cm^2 of UVB exposure, triple the minimal erythema dose, (c) exposure to milk phospholipids in the media, and (d) exposure to milk phospholipids in the media followed by exposure to UV light. Specimens were imaged directly after exposure, 24 hours after exposure, and 48 hours after exposure. The results suggest that milk phospholipids act upon skin cells in a protective manner against the effect of ultraviolet radiation. Preliminary experiments determining the mechanisms by which the benefits occur are underway.

3303-Pos Board B350

Biomineralization By The Marine Tubeworm Hydroides Dianthus: Structure And Composition Of The Adhesive Cement

Adrienne E. Tanur, Nikhil A. Gunari, A. Sullan Ruby May, Christopher J. Kavanagh, Gilbert C. Walker.
University of Toronto, Toronto, ON, Canada.

The structure and composition of the adhesive cement of Hydroides Dianthus was studied using a variety of characterization techniques, including XRD, FTIR, SEM, EDX, and AFM. The cement was determined to be a composite of inorganic CaCO3 crystals in an organic matrix, with the organic component making up only a small fraction of the material. Two polymorphs of CaCO3, in roughly equal proportions, were identified in both the tube shell and the cement via XRD and FTIR: aragonite (CaCO3), and magnesium calcite ((Ca,Mg)CO3). Electron microprobe and EDX measurements also confirmed the presence of magnesium. SEM imaging revealed two distinct crystal habits, and EDX measurements allowed for the identification of crystals with an acicular habit as aragonite, and crystals exhibiting a triangular layered structure as magnesium calcite. AFM measurements in sea water and in air were performed in order to determine the elastic moduli of the various components of the composite cement. For the inorganic component, moduli in the range of ~3 GPa were observed in the wet state, and values in the range of ~11 GPa were